A COMPILATION OF CRITICAL SCIENCE QUESTIONS

The following critical questions are compiled from HQ Discipline Science Plans and early Advisory Committee Reports, as they relate to Ames life sciences activities, and the Space Station Biological Research Project critical questions (provided by C. Wade for Cell, Developmental and Plant Biology disciplines only). When possible, questions for SSBRP (in italics, whose numerical reference reflects its position in the original document) are linked to similar questions from the HQ discipline plans and Advisory Committee list.

The most recent Advisory Committee report (1998) from the Space Studies Board, "A Strategy for Research in Space Biology and Medicine in the New Century", Osborne, Chairman, contains recommendations for research by discipline based on findings to date and the development of new ground-based technologies and methodologies that make new avenues of research possible. Recommendations presented by discipline, that pertain to the research focus at Ames, are included below before the individual critical questions (taken from the Paine (1986), Ride (1987), Goldberg (1987), Robbins (1988), Augustine (1990), Lanzerotti (1991), and Vison 21 (1992), reports). The Osborne report also contains a chapter focused on prioritization of research efforts based on two criteria: 1) ensuring health and survival of astronauts after prolonged exposure to spaceflight; and, 2) conducting studies in fundamental biological processes that are impacted by gravity. These recommendations that pertain to non-human life sciences research, in order of priority, are presented at the end of this report.

Behavior, Performance and Human Factors

NRC SSB 98/Recommendations:

? ? Ground-based studies of change and stability in individual physiological patterns (e.g., cardiovascular, neuroendocrine, and immune system changes related to alterations in the hypothalamic-pituitary-adrenal axis) in response to psychosocial and environmental stress and their applicability to measures of behavior and performance in flight and;

? ? Development of psychophysiological instrumentation that is highly portable, nonintrusive, and robust in the space environment.

1. How does prolonged spaceflight affect behavior and group dynamics (including species, sex, and age differences)?

2. How do physiological changes induced by the space environment affect behavior, performance, perception and cognitive functions?

3. To what extent do circadian rhythms and sleep influence performance in space?

Cardiopulmonary: Cardiovascular

NRC SSB 98/Recommendations:

- ? ? Investigate the specific mechanisms underlying the inadequate total peripheral resistance during orthostatic stress observed postflight, and determine the effective countermeasures. Both human and instrumented animal models will be required.
- ? ? Determine the appropriate method for referencing intrathoracic vascular pressures to systemic pressures, given the observed changes in alveolar and thoracic volume and compliance in microgravity.
- ? ? Determine the nature, magnitude, and time course of cardiovascular adjustments to microgravity, including assessment of hemodynamic, neurohumoral, morphological, histological, and molecular changes in the cardiovascular system.
- ? ? Determine whether microgravity-induced changes in local perfusion cause changes in vascular or vasomotor control (vascular proliferation or atrophy, secretion of endothelially derived vasoactive substances and microcirculatory autoregulatory mechanisms, and so on) or organ function (pulmonary gas exchange, renal clearance mechanisms, blood-brain barrier and cerebral pressure, etc.)
- ? ? Identify and validate countermeasures that might be effective in combating long-duration space-flight-associated cardiovascular changes, and determine the mechanisms by which changes in fluid distribution and metabolism affect countermeasures designed to protect against cardiovascular and musculoskeletal deconditioning (e.g., LBNP, fluid rehydration, centrifugation, exercise, drugs, etc.).
- ? ? Determine the relationship between the cardiovascular adjustments to spaceflight and those occurring in Earth-based models.
- ? ? Determine the relationship of cardiovascular adjustments to microgravity-induced changes in other systems, especially the neurovestibular, autonomic nervous, hematopoietic (blood forming), and renal-endocrine systems.
- ? ? Determine whether there are changes in myocardial function associated with microgravity (contractile proteins, excitation-contraction coupling mechanisms, cardiac energetics, atrophy, etc.)
- ? ? Determine whether there are additional uses of microgravity for the study of terrestrial cardiovascular processes.

1. What are the specific mechanisms underlying postflight orthostatic intolerance and what are the appropriate countermeasures?

2. What are the underlying mechanisms of spaceflight-induced effects on basic cardiovascular function?

3. What are the appropriate countermeasures used to combat cardiovascular deconditioning in short and long-duration flight and how should they be applied (i.e., LBNP, fluid anti-g rehydration, centrifugation and exercise)?

4 How appropriately do Earth-based simulation models such as bed rest, immersion, head-down tilt, and physical unloading represent the effects experienced in spaceflight? Are there appropriate animal models for studying the functional elements of cardiovascular adjustments to microgravity?

5. How will long-term spaceflight affect cardiac function? Will there be irreversible, involutional myocardial degeneration or "hypotrophy"?

6. What are the delayed or persistent consequences following long-term exposure to microgravity?

7. How can studies using microgravity contribute to better understanding of cardiovascular function on Earth?

Cardiopulmonary: Pulmonary Function

NRC SSB 98/Recommendations:

- ? ? Determine how microgravity alters lung deposition of aerosol and whether this constitutes a health hazard.
  - ? ? Does the absence of the sedimentation mechanism cause deeper penetration of particles into the lung?
  - ? ? What is the aerosol concentration , particle size profile, and bacterial contamination in the current spacecraft environments and atmospheres?

? ? Does microgravity alter nonventilatory responses to aerosolized antigens (immune responses, phagocytosis, etc.)?
? ? Does long-duration exposure to microgravity affect pulmonary aging or disease processes?
? ? Are pulmonary function changes associated with long-duration exposure to microgravity different from those seen in short (<1 month) flights?
? ? Determine whether lunar or martian dust particles are associated with mechanical, allergic, or biochemical pulmonary toxicity.
? ? Determine whether respiratory muscle structure and function deteriorate in weightlessness and, if so, whether the changes decrease maximal oxygen uptake.
? ? Determine and characterize intrathoracic lymph flow, and changes in blood pressure and volume associated with microgravity.

1. Does the environment of space microgravity pose a particular health risk to pulmonary function (e.g., significant changes to lung volume, rib cage, diaphragm, and abdominal wall configurations, as well as changes in blood flow, ventilation, alveolar size, intrapleural pressures, and respiratory control mechanisms)?

2. Is pulmonary function altered in long-duration spaceflight?

3. How appropriately do Earth-based simulation models such as bed rest, immersion, and head-down tilt represent the effects experienced in spaceflight? Are there appropriate animal models for studying the functional elements of pulmonary adjustments to microgravity?

4. What are the risks to proper pulmonary function associated with various EVA maneuvers and what are the appropriate life support procedures for protection and resuscitation of crewmembers in the event of loss of pressure?

Cell Biology

<u>NRC SSB 98/Recommendations:</u>
? ? Studies of mechanisms of cellular mechanoreception should include identification of the cellular receptor, investigation of possible changes in membrane and cytoskeletal architecture, and analysis of pathways of response, including signal transduction and resolution in time and space of possible ion transients.
? ? Studies of cellular responses to environmental stresses encountered in spaceflight (e.g., anoxia, temperature shock, vibration) should include investigation of the nature of cellular receptors, signal transduction pathways, changes in gene expression, and identification and structure and function analysis of stress proteins that mediate the response.
? ? NASA Should work with the scientific community and industry to foster development of advanced instrumentation and methodologies for space-based studies at the cellular level.

1. How do single cells detect gravity?

1. *Are single cells capable of sensing and responding to changes in gravity, or are only multicellular systems capable? Do single cells "sense" gravity directly (intracellularly) or indirectly (environmentally-mediated effect)?*

2. *If single cells are too small to detect changes in the gravitational field directly, what are the environmental changes responsible for the cells' response? Is the cessation of microconvection currents at microgravity responsible?*

3. *Do single cells sense alterations in gravity directly, in which cells are part of a gravisensing organ, or indirectly, in which the cells detect indirect consequences of the presence or absence of inertial acceleration?*

4. *How do the following modifying factors affect gravity "sensing" at the cell level: cell size, cellular dynamics; changes in cell shape; prokaryotic versus eukaryotic cells adaptive versus non-adaptive cells; circadian rhythms?*

2. How are cell functions influenced by gravity?

5. *If single cells sense changes in gravity directly, what are the intracellular structural/functional mechanisms that are sensitive to gravity perturbation? Is the cytoskeleton organization of cells disturbed by gravity perturbation? How does the cell's cytoskeleton, outer membrane and nuclear envelope/nuclear matrix react to altered gravity, as a three-dimensional continuum of perception and structural integrity?*

6. *If multicellular systems are necessary for gravity sensing, how is this effected? What cellular structures and processes that extend across several cells might be involved? What aspects of cell-cell communication are affected? Would the requirements for cellular interaction/assembly increase sensitivity to indirect or environmentally mediated effects (e.g., reduction of cell-cell and cell-surface contact by dispersion of cells in microgravity)?*

7. *How are the following cell functions influenced by gravity and/or affected by microgravity: the expression and regulation of genetic information; cell division; cell differentiation; signal transduction, including signal-membrane interactions, membrane-effector interaction, and signal-effector linkage; membrane dynamics; intracellular transport; secretion; alternate pathway regulation; and cell-to-cell communication? The importance of selecting cells and cell lines that can provide interpretable results bearing on precise questions cannot be overemphasized.*

8. *How long can single cells cope with changes in gravitational force without adverse results? Do these effects persist after return to unit gravity?*

3. How do cells transduce gravitational stimuli?

9. *What are the mechanisms involved in the transduction of the stimulus of altered gravitational force to a cellular response? By what pathways is the perception of altered gravity relayed intracellularly and/or extracellularly?*

10. *Research indicates that resting/native cells are not measurably affected by changes in gravity. What is responsible for the difference in responsiveness between resting and active cells?*

11. *How does the gravity stimulus affect cellular responses following the binding of specific growth factors to their cognate membrane receptors--as an independent variable or a quantifier? What are the contributions of the cytoskeleton, the intracellular pathways of chemically mediated signal transfer, and the nuclear envelope/nuclear matrix to functional response?*

12. *How will altered gravitational fields and vectors change the information content of the three-dimensional microenvironment of the cells (stroma and matrix connections)? How does microgravity affect these signals under both homeostasis and challenge?*

13. *How are cell-cell and cell-surface contacts in multicellular systems affected by microgravity?*

14. When do gravitational effects appear? Are there differences between responses that occur as a direct consequence of acute exposure to microgravity and responses at a later time, that may reflect the operation of compensatory mechanisms?.

Other

4. What model cell systems can be used to identify processes and mechanisms by which cells respond to altered gravitational force?

15. How is the effect of gravity (and microgravity) on cells influenced by magnetic fields and radiation?

16. How can gravity be used as a research tool in perturbing cell structure/function in the absence of other effectors?

17. To take the best advantage of flight opportunities, how can life science experiments on cellular systems be automated in protocol, remote manipulation, and data retrieval and analysis?

Controlled Ecological Life Support System (CELSS)

1. What is the productivity, transpiration, dry matter partitioning, morphology, and reproductive capability of plants at less than 1-g?

2. What is the maximum productivity, in terms of edible biomass, of crop plants grown extra-terrestrially? And what are the conditions necessary to sustain maximum yield in a less than 1-g environment?

3. What genetic technology can be used to develop crop cultivars better fit for CELSS use in space?

Developmental Biology

NRC SSB 98/Recommendations:

? ? Key model organisms should be grown through two complete life cycles in space to determine whether there are any critical events during development that are affected by space conditions. Because no critical effects have been seen in model invertebrates, the highest priority should be given to testing vertebrate models such as fish, birds, and small mammals such as mice or rats. If developmental effects are detected, control experiments must be performed on the ground and in space, including the use of a space-based 1-g centrifuge, to identify whether gravity or some other element of the space environment induces the developmental abnormalities.

Note: This recommendation is given high priority by the Board (see last section).

? ? Studies should be performed to identify the critical periods in vestibular neuron development, including proliferation, migration, differentiation, and programmed cell death. This information is essential to design and interpret subsequent flight experiments on the effects of microgravity on vestibular development.

? ? The role of otolithic stimulation on the development and maintenance of the different neural space maps – including those within the brainstem, hippocampus, sensory and motor cortices, and corpus striatum – should be investigated.

? ? Studies should be designed to address how neurons of the various sensory and motor systems interact with vestibular neurons in the normal assembly and function of the neural space maps.

? ? Studies should be performed to determine the influence of decreased stimulus (as experienced in microgravity) on the development and maintenance of the neural space maps.

? ? From ground-based studies, researchers know that there are compensatory mechanisms that function normally in the vestibulomotor pathways, and these mechanisms occur in space. What is the basis for the compensation on Earth and in space, and are the mechanisms the same? These experiments should be given the highest priority, because these compensatory mechanisms operate in astronauts entering and returning from space and may have a profound effect on their performance in space and their postflight recovery on Earth.

Note: This recommendation is given high priority by the Board (see last section).

? ? Experiments are needed to critically test the role of gravity on the development and maintenance of the vestibular system's capability for neuroplasticity. The process needs to be characterized at several different times following perturbations to determine the sequence of intermediate events leading to the plastic change. Controls for the effects of nongravitational stresses likely to be encountered in space (such as loud noise and vibration) must also be performed on the ground, so that space experiments can be designed to isolate the effects of microgravity from the effects of other stresses.

Note: This recommendation is given high priority by the Board (see last section).

? ? From ground-based experiments, the vestibulo-oculomotor system is known to be capable of learning new motor patterns in response to sensory perturbations. Future investigation should focus on determining if and how these mechanisms are affected by exposure to microgravity.

1. What is the role of gravity in the various stages of animal development (i.e., fertilization, gametogenesis, organogenesis, anatomical structure development and growth)?

1. How does gravity affect organogenesis and the development of anatomical structures?

2. What will be the result of gravity-induced dys-synchrony (temporal or hormonal) during embryonic development?

3. Which developmental mechanisms have evolved to be dependent on the 1-g gravity field and vector?

4. What events in gametogenesis and early germ cell maturation are gravity sensitive, and how can these results relate to the proliferation and differentiation of other individual cell types?

5. Can altered gravities affect fertilization, and do these results indicate more general mechanisms of membrane alteration in individual cells?

6. Which responses are transmitted maternally and which are intrinsic to the developing embryo?

7. What are the results of altered gravity fields on the axis polarity and symmetries of the zygote?

8. Are there gravity effects that can terminate in changes of gene activation?

9. What structural and morphometric alterations will occur in the extracellular matrix, the connective tissue, and the musculoskeletal systems? How will this result in altered differentiation of cells, and in changed tissue composition?

2. What are the joint effects of radiation and microgravity?

10. What are the joint effects of radiation and microgravity?

3. Which developmental mechanisms and organ systems are dependent on the 1-g gravity field and vector?

11. Which organ systems are dependent on the 1-g gravity field and vector.

4. How does long-term exposure to microgravity affect reproductive cycling and spermatogenesis?

12. What are the effects of gravity, in concert particularly with life in closed ecosystems, on sexual maturation?

5. What is the effect of altered gravity on mammalian mating behavior and parent-young interactions?

13. How will gravitational fields, particularly microgravity, disturb the precise coordination and postural control required in mating?

14. How will parent-young interactions be altered in the space environment?

15. What will be the effects on lactation, suckling and related parent-young bonding mechanisms?

6. How does altered gravity affect genotype expression?

16. How do specific organs and tissues respond developmentally to altered gravity, as demonstrated by the expression of selected target genes in transgenic mice with pre-determined genetic makeups?

7. How does gravity produce responses in cultured cells that mimic those seen in chronologically aged cells, those isolated from accelerated aging syndromes, and senescent cells in-vitro?

17. How does gravity produce responses in cultured cells that mimic those seen in chronologically aged cells, those isolated from accelerated aging syndromes, and senescent cells in vitro?

18. Which de-limiters of lifespan have relevance to gravitational effects?

19. How do neoplasms common to chronological aging relate to limitations of cell lifespan and susceptibility to abnormal growth regulation under altered gravitational fields?

8. How does development in an altered gravity environment affect the ability of the organism to mature and reproduce in the altered environment and upon return to a 1-g environment?

20. Does the developmental ontogeny of animals raised through more than one life cycle under a changed gravity field differ from the 1-g classical pattern? Does this altered pattern reside in the genome, or is it relayed from hormonal and stromal interactions?

Other

21. Will hatching or parturition occur normally?

22. In the period of rapid-natal growth, which systems are the most sensitive to altered gravity perturbations?

23. Are the gravity sensitive systems (i.e., vestibular, proprioceptive, cardiovascular, musculoskeletal) of young and adult animals similarly sensitive to this stimulus in ontogeny?

24. At what developmental stage can we observe perturbations of circadian rhythms, both temporally and with respect to differentiation state?

25. Are there critical windows of susceptibility for developmental processes, or is development affected in a gradient?

26. If gravity related effects exist, can they be reversed in the short or long term?

27. Considering development as a series of stages or phases, beginning with pattern specification and progressing through differentiation, how will gravity affect selected phases in animals that represent different species and phyla?

28 . *Will aquatic animals perceive and respond to gravity as do their terrestrial counterparts? Those animals which pursue different life states in both environments may be particularly valuable for study.*

Environmental

1. What impact do spaceflight-induced changes (biological, physiological, and immunological) have on the susceptibility of crewmembers to toxic materials?

2. What is the effect of spaceflight on microorganisms (e.g., environmental microflora)?

Exobiology

1. What was the path by which the ancestors of modern microbes evolved from the first living organisms and how did the evolving biota modify their environment over time?

2. What was the nature of the earliest forms of life, and in what sequence were new attributes acquired?

3. What is the relationship between biological evolution and changes in the environment?

4. What are the simplest biochemical mechanisms and biophysical structures that can fulfill the functions of living systems, and what irreducible combinations of these constitute a living entity?

Immunology

NRC SSB 98/Recommendations: Animal Studies listed in priority

? ? Resistance to infection. Studies of resistance to infection might create some difficulty if carried out entirely in the space station environment, because of the difficulty in bringing potential pathogens into that environment. Therefore, studies of infection should not be carried out on board the space station or the space shuttle but should be performed on animals flown in space immediately upon their return to Earth. This would model the period immediately after return to Earth when crews leave closed environments, which could be the time of crew members' greatest risk from infection. All other animal studies should be carried out both on the space station and upon return to Earth. Animals exposed to the spaceflight conditions or suspension modeling for short- or long-term periods should be infected with viruses (e.g., influenza and herpes viruses) or bacteria (e.g., *Salmonellla typhimurium*) and spaceflight-induced changes in resistance to infection determined. These would test the relevance of spaceflight effects on both antibody and cell-mediated immunity.

? ? Acquired immune responses

? ? Studies of antigen sensitization and resistance to infections should be carried out to determine if spaceflight affects the ability to mount a new immune response and resistance to infection. Sensitization studies could be carried out in flight using innocuous sensitizing antigens such as keyhole limpet hemocyanin.

? ? Hummoral immune responses should be tested directly after immunization and challenge by determination of antibody specificity and type. Th1 and Th2 cytokine profiles should be established not only in nonimmune animals but also following specific immune challenge.

? ? Cell-mediated immunity should be tested with both delayed-type hypersensitivity responses to contact-sensitivity agents, and generation of cytotoxic T lymphocyte activity.

? ? Interactions with the HPA-axis and other body systems. When measurements are taken for immune responses, simultaneous determination of stress-related mediators (including stress hormones, catecholamines, and neuropeptide Y) should be carried out. Measurements of blood pressure, heart rate, orthostatic intolerance, and other appropriate variables/parameters should also be collated and made available to allow determination of interactions of the immune systems with other body systems, including the musculoskeletal system.

Note: This recommendation is given highest priroity by the Board (see last section).

1. What are the effects of spaceflight on the lymphoid organs - nodes, spleen, thymus, and bone marrow?
2. What are the chronic effects of long-term exposure to space in terms of resistance to disease and tumor development?
3. How do changes in the particular subcomponents of the immune system affect its overall function in microgravity and upon return to Earth?
4. Are there in-vitro tests that reliably predict decreases in immune function in spaceflight? And are there appropriate ground models, human, animal or computer, that simulate the effects of spaceflight with regard to the immune system in space?
5. What are relationships between spaceflight stressors and immune function and are there effective countermeasures?

Musculoskeletal: Bone and Mineral Metabolism

NRC SSB 98/Recommendations: Animal Studies

? ? Determine if changes produced by microgravity in animal bones are similar to those in humans and if they have a similar basis – that is, increased bone resorption and/or decreased formation.
- ? ? Older animals (rats and mice) should be used (the growth in rats is significantly attenuated at about 12 to 14 months). Mice are preferable, because they are smaller and include genetically homogeneous strains, mutants, and transgenic animals ("knockouts", and so on).
- ? ? Dual x-ray absorptiometry, histomorphometry, and biochemical markers should be used.

? ? When an animal model is identified that mimics human changes in spaceflight, it should be compared to ground-based models, such as hindlimb unloading. If appropriate, it should be used to study mechanisms that can then be corroborated in space. Emphasis should be on investigations that can help develop countermeasures and shed light on human pathology (e.g., osteoporosis).

Note: This recommendation is given high priority by the Board (see last section).

? ? Studies should be conducted that will do the following:
- ? ? Evaluate the contribution of changes in muscle function and blood flow. Muscle, bone, and blood flow, which strongly affect both, should be studied side by side. Given the dynamic nature of the vascular system, in-flight experiments should include rapid tissue fixation to best address this question.
- ? ? Evaluate to what extent the bone loss is secondary to systemic effects on hormones, growth factors, and cytokines, which should be measured in the same experiments. Identify putative mediators involved in the tissue response: prostaglandins (PGE2, PGI2), growth factors (FGF, TGFB), and so on. Recombinant proteins and mutated cells and animals can supplement a pharmacological approach.
- ? ? Identify the cells responsive to gravity changes (osteoblasts, osteocytes, osteoclasts) and determine at what level the cellular processes are altered: cell recruitment, cell activity, cell survival (aptosis). The questions can be addressed by the combination of in vivo (histology) and in vitro (cell biology) methods.
- ? ? Evaluate the interactions between osteoblast lineage cells and osteoclast precursors under microgravity conditions with 1-g centrifuge controls, using the well-established ground-based experimental models. Examine if production of factors known to mediate the communication between osteoblasts and osteoclasts (such as prostaglandins, inflammatory cytokines, hemopoietic growth factors, matrix molecules, and others) are altered under microgravity conditions.
- ? ? Examine whether bone cells, or cells that convey the information to bone cells, respond to (a) strain in the matrix to which they are attached, (b) shear stress produced by fluid flow, and/or (c) electrical fields produced by deformation of fluid flow. This should be investigated using primarily ground-based experiments, taking

advantage of the potential of current cellular and molecular biology methods (transgenic animals and the like). Identify the sensors for these perturbations (e.g., integrins, ion channels), and determine the pathways for their signal transduction and amplification.

? ? Determine if there are molecular alterations in the structure of bone matrix or bone mineral.

? ? Examine the composition of the matrix and the presence of posttranslational modifications (e.g., collagen cross-links). The discovery of new proteins and their secondary modifications warrants reexamination of this question in the context of mechanical and gravitational changes.

? ? Determine if there are changes in mineral structure (crystal size and composition), the degree of mineralization, or relationship of the bone mineral to the bone matrix.

1. What is the sequence and time-course for changes that occur in bone, bone matrix, and connective tissue for different areas of the body as a result of exposure to short and long duration space microgravity and simulated microgravity? What are the changes on a local basis, in stresses, strains, and strain rates?

2. What is the level and course of reversibility of bone and connective tissue changes upon return to 1-g environment?

3. What are the key cellular and extracellular processes that are required to maintain healthy bone metabolism? How are these processes influenced by microgravity?

4. What are the risks to bone fracture healing in space given the observed altered bone metabolism and changes in bone matrix? Is the initial deposition of cartilage following bone fracture in microgravity eventually replaced by bone as occurs on Earth or does it deviate, resulting in weaker tissue?

5. What are specific countermeasures to prevent deleterious changes to bone and connective tissue and ensure proper fracture healing?

6. Are there microgravity-induced gender differences in bone metabolism? And, if so, what are the underlying mechanisms?

Musculoskeletal: Muscle

NRC SSB 98/Recommendations:

? ? The efficacy of muscle repair in microgravity should be more thoroughly examined at the sarcomere, myofiber, motor neurons, and tissue levels.

? ? Myotendinous junctions as structural failure sites warrant further study to prevent tendon rupture during transition to gravity reloading.

Development of improved countermeasures is a high-priority goal, and should include the following: **Note: This recommendation is given high priority by the Board (see last section).**

? ? Consideration of the potential value of hormones, growth factors, second messengers, and drugs in the design of novel, more effective countermeasures; and

? ? Improved understanding of mechanisms of microcirculation failure, which is required for design of in-flight countermeasures for preventing reloading damage.

? ? The process of transitioning from microgravity to higher gravity without undue damage and loss of performance needs to be explored by examination of microgravity-adapted individuals transitioning to 1 g and 1-g adapted individuals exposed to hypergravity by centrifugation.

? ? NASA should actively work with the scientific community and industry to develop equipment to reduce in-flight muscle stress and fatigue as well as instrumentation necessary for in-flight experimentation and monitoring of muscle function and health. Examples include the following:

- ? ? Rapid freezing apparatus to preserve biospecimens obtained in flight for later biochemical and molecular assays; and,
- ? ? Dual-beam x-ray and ultrasonography for noninvasive monitoring of muscle deterioration and real-time assessment of the efficacy of countermeasures.

? ? The highest priority for research projects should be those investigations designed to elucidate the cellular and molecular mechanisms underlying muscle weakness, fatigue, incoordination, and delayed-onset muscle soreness. These deficits are associated with muscle cell atrophy, greater susceptibility of muscle fibers to contraction-induced destruction of fibers, and a compromised microcirculation leading to ischemic necrosis and secondary changes in muscle tissue.

? ? Ground-based models such as bed rest for humans and hindlimb unloading for rats should continue to be used for testing and refining hypotheses seeking to understand the fundamental mechanisms of how workload is transduced into molecular signals regulating muscle mass and protein isoform expression, as well as the cellular and molecular control myofilament assembly and length. Integrated studies employing multiple intra- and interdisciplinary approaches are encouraged.

? ? Fundamental work is needed on myogenesis, fiber type differentiation, neuromuscular development, and spinal development using neonatal rodents as model systems. The mechanisms should be determined whereby muscle cells sense working length and the mechanical stress of gravity. Signal transduction pathways for growth factors, stretch-activated ion channels, regulators of protein synthesis, and interactions of extracellular matrix and membrane proteins with cytoskeleton should be investigated.

? ? In-flight and hindlimb unloading testing of genetically engineered murine models with gain and loss of function, up regulation and down regulation of key proteins, and release of candidate hormones and growth factors and their respective receptors should be exploited for probing basic mechanisms of neuromuscular function during adaptation to unique hypo- and hypergravity environments.

1. What is the time course and extent of muscle atrophy during space and simulated microgravity?

2. What are the functional effects for both skeletal and smooth muscle changes?

3. What is the relationship between muscle activity and bone function and morphology (i.e. biomechanics)?

4. How is muscle metabolism regulated during normal activity and exercise, after acute and long term microgravity or unloaded conditions, and during recovery from microgravity or unloading?

5. What is the level and course of reversibility of muscle atrophy, structural change, fiber type transformation?

6. Is there an appropriate ground model for simulating the changes in muscle that occur in space?

7. Can injured muscle be repaired in microgravity?

8. What are the appropriate countermeasures to ensure adequate muscle structure, function and repair capacity?

Neuroscience: Central Processing

NRC SSB 98/Recommendations:

? ? Bed rest studies may serve as useful models for inducing sensory-motor reorganizations.

? ? Test strategies should be developed to determine the sensorimotor and cognitive consequences of central nervous system reorganizations resulting from exposure to microgravity and their implications for reentry disturbances.

? ? The relationship of motion sickness to altered sensorimotor control of the head and body as a function of altered force background and effective body weight should be assessed.

? ? The possibility of maintaining dual adaptations to more than one force background simultaneously – allowing transitions between them without performance decrements – should be explored.

- ? ? Combined physi9ological and morphological studies using animal models should be performed to investigate the interaction between the vestibular system and autonomic function, including cardiovascular regulation.
- ? ? Testable models should be developed that integrate current knowledge of terrestrial and space motion sickness.

1. What are the changes in the processing of signals from the vestibular end organs and where do these changes take place (i.e., within the vestibular nuclei, cerebellar structures or other related brainstem and cortical structures)?

2. What are the underlying mechanisms contributing to SMS?

3. How can studies using the microgravity environment of space contribute to better understanding of vestibular-related dysfunctions on Earth?

4. Does altered gravity lead to changes in neural control of biological rhythms?

? ?What is the neural basis for the adaptive response to altered sensory environments?

Neuroscience: Motor

NRC SSB 98/Recommendations:

- ? ? Animal models of reentry disturbances could be developed to elucidate the underlying physiological processes of postural and locomotory adaptations.

1. What are the characteristics of gaze and eye-head coordination, posture and locomotion in altered gravity environment

2. What are the optimal procedures for readaptation to 1-g following adaptation to microgravity?

3. What appropriate ground models can be used to accurately predict motor behavior in altered gravitational states?

4. What are the appropriate countermeasures to prevent postflight motor dysfunction?

Neuroscience: Cognitive/Spatial Orientation

NRC SSB 98/Recommendations:

- ? ? There is a critical need to evaluate the influence of microgravity and other non-1-g force levels on the integrative coordination of complex body activities, including reaching and locomotory movements involving combinations of eye, head, torso, arm and leg activity. Studies should be performed before, during, and after spaceflight so that initial disruptions and the time course of adaptation and readaptation can be identified.
- ? ? It is important for the success of long-duration space missions to determine the sensory, motor, and cognitive factors influencing the ability to adapt and to related adaptation to different force backgrounds. These experiments can be conducted on the ISS, in rotating environments, and in parabolic flight.
- ? ? Neural coding of spatial navigation may be affected by a change from a 1-g to a microgravity force background and may relate to some of the orientation illusions experienced by astronauts. The influence of altered force levels on orientation and geographical localization should be explored in parallel experiments with humans and animals. Ground-base centrifuges, body-loading paradigms, parabolic flight conditions, virtual reality conditions, and eventually orbital flight should be utilized for testing.

1. What are the underlying mechanisms of motion perception and spatial orientation?

2. How does vestibular input modify visual and auditory localization and multisensory spatial orientation?

3. What ground-based simulation models are most effective in evaluating interactions of angular and linear acceleration, proprioception, somatosensory and visual inputs in determining orientation in a three-dimensional environment and how are these interactions changed in altered gravity?

4. What are the appropriate countermeasures to prevent postflight disequilibrium?

Neuroscience: Sensory Receptors

NRC SSB 98/Recommendations:

? ? The effect of altered calcium regulation in microgravity on otoconial development and regeneration should be determined using animal models.

? ? In-flight electrophysiological recordings of otolith afferent and efferent activity and signal processing within the brain should be made in test animals.

1. What are the structure-function relationships of the otolith organs and semicircular canals?

2. What are the mechanisms of vestibular hair cell transduction and transmission?

3. How will the chronic decrease in afferent input to the vestibular, proprioceptive and somato-sensory systems from long-duration flight affect reflexes?

4. What are the relative sensors for posture, body movement, and spatial orientation, including transduction?

Plant Biology: Perception, Transduction and Response

NRC SSB 98/Recommendations:

? ? The Advanced Life Support (ALS) program should concentrate its ground-based research on developing a completely enclosed plant growth system. This effort will require close collaboration between engineers and plant environmental scientists.

? ? The ALS spaceflight program should focus on testing the potentially gravity-sensitive components of the closed plant growth system, such as the nutrient delivery system.

? ? A lower priority concern is a ground-based study of the problems and mechanisms associated with stresses that plants in space might encounter, including super-high CO_2 levels and vibrations.

? ?A primary focus of NASA-sponsored research in plant biology should be on the mechanisms of gravitropism. In particular, modern cell and molecular techniques should be used to determine the following:

? ? The identity of the cells that actually perceive gravity, and the role of the cytoskeleton in the process;

? ? The nature of the cellular asymmetry set up in a cell that perceives the direction of the gravity vector;

? ? The nature and mechanism of the translocation of the signals that pass from the site of perception to the site of reaction; and,

? ? The nature of the response to the signal(s) that leads to alterations in the rate of cell enlargement.

Note: The previous recommendations are given high priority by the Board (see last section).

? ? Maximum use should be made of mutants and gene sequencing to identify specific proteins involved in gravitropism.

? ? A secondary focus should be on the mechanisms of graviperception and graviresponse in single cells, especially the algae and mosses.

? ? As a lower priority, NASA should consider supporting research on algal gravitaxis.

1. What are the basic mechanisms whereby plants perceive, transduce, and respond to a gravitational force?

1. What are the mechanisms that underlie gravity perception?

2. What are the sequential events in gravity transduction and response?

3. What changes in the routes of perception, transduction and response occur in microgravity?

2. What are the gravity thresholds?

4. What are the thresholds required for gravity to have an effect?

3. What are the species-specific differences, if any, in perception and response to gravity?

5. What are the differences, if any, between species and their tissues in their perception and responses to gravity?

4. How does a single cell sense gravity?

6. How do single cells sense gravity?

Plant Biology: Reproduction and Development

NRC SSB 98/Recommendations:

? ? The seed-to-seed experiment, using *Arapidopsis thaliana* and *Brassica rapa* plants, should be top priority for the ISS. This experiment must be conducted on the ISS, because the plants should be grown through at least two generations in space.

? ? To conduct a meaningful seed-to-seed experiment, NASA needs to develop the following:

? ? A superior plant growth unit, with adequate lighting, gas exchange, and water and/or nutrient delivery; and

? ? Arabidopsis thaliana plants that are insensitive to expected environmental stresses (such as ethylene) and that contain indicator genes for all the expected environmental stresses, such as high levels of CO_2, vibration, anaerobiosis, water stress, and temperature stresses.

7. In the interim, before the ISS is functional, studies on specific stages of plant development in space should be limited to small plants with short life cycles (e.g. *Arapidopsis thaliana* or *Brassica rapa)*. Whenever possible, a 1-g on-board centrifuge should be available.

Note: These last 3 recommendations are given high priority by the Board (see last section).

1. What is the role of gravity in the development and reproductive processes of plants?

1. Can plants successfully reproduce through more than one generation in space?

2. Is chromosomal integrity and behavior during cell division affected in microgravity?

3. Is cell, tissue, or organ differentiation affected in microgravity?

4. What effect does microgravity have on embryogenesis and the ensuing stages of the life cycle of plants from maturity to flowering and senescence?

5. Are microgravity-grown tissues and organs competent?

2. How do plants adapt to microgravity?

6. How do plants adapt to microgravity?

3. What are the interactive effects of microgravity and radiation on plants?

7. Are there unique interactions between space radiation (or other environmental factors) and microgravity that affect the development of biological systems in space?

Other

8. Are the growth rates of higher plants or single cells affected by microgravity?

Plant Biology : Metabolism and Transport

1. What is the role of gravity in metabolism, photosynthesis, and transport processes in plants?

1. Are anabolic and catabolic pathways and the photosynthetic apparatus and pathway altered in microgravity?

2. What effect does microgravity have on the synthesis of storage and support polymers?

3. What are the effects of the space environment on membranes and transport during uptake and secretion?

4. Are pathways for nutrient absorption altered in microgravity?

5. What are the effects of the space environment on long distance transport of water and on transpiration?

6. What are the mechanisms by which transport systems are polarized in plants grown in space?

Radiation Biology

NRC SSB 98/Recommendations:

? ? Determine the carcinogenic risks following irradiation by protons and HZE particles.

Note: This recommendation is given high priority by the Board (see last section).

? ? Can the risk due to irradiation by protons in the energy range of the space environment be predicted on the basis of the risk posed by exposure to low-LET radiation, such as gamma rays, and is there evidence for the repair of damage in cells following fractionated exposure to protons and HZE particles?

? ? What are the appropriate RBEs for HZE particles?

? ? Determine how cell killing and induction of chromosomal aberrations vary as a function of the thickness and composition of shielding.

? ? Determine whether there are studies that can be conducted to increase the confidence of extrapolation from rodents to humans of radiation-induced genetic alterations that in turn could enhance similar extrapolations for cancer.

? ? Determine if exposure to heavy ions at the level that would occur during deep-space missions of long duration poses a risk to the integrity and function of the central nervous system.

Note: This recommendation is given high priority by the Board (see last section).

? ? Determine if better error analyses can be performed of all factors contributing to the estimation of a risk by a particular method, and determine the types and magnitude of uncertainty associated with each method. What alternate methods for calculation of risk can be used for comparison with conventional predictions in order to assess absolute uncertainties? How can these methods be used to better determine how the uncertainties in the methods affects estimates of risk to humans and estimates of mission costs?

Lower priority recommendations:

- ? ? Estimate the risks of reduced fertility and sterility as a result of exposure to radiation on missions of long duration in deep space.
- ? ? Estimate the risks of clinically significant cataracts being induced by exposure to radiation at the levels that will occur on extended spaceflights.
- ? ? Determine whether drugs can be used to protect against the acute or carcinogenic effects of exposure to radiation in space.
- ? ? Determine if there is an assay that can provide information on an individual's sensitivity to radiation-induced mutagenicity and that also can be predictive of a predisposition for susceptibility to cancer.
- ? ? Determine if there are differences in biological response arising from exposure to particles with similar LET, but with different atomic numbers and energies.

1. What are the effects of spaceflight radiation on male and female reproduction and the possible pathological effects of this to eggs, sperm, and developing embryos?

2. How can animals be used to extrapolate probabilities of radiation risk to humans in space?

3. How are atomic and nuclear interactions related to biological effect?

4. How accurate are ground-based models in predicting the effects of space radiation on living organisms?

5. What are the effective countermeasures, other than shielding, to radiation-induced damage of cells, tissues and animals?

6. How can cellular and molecular mechanisms of radiation damage be used to understand effects in whole cells and organisms?

Regulatory: Circadian Rhythms

1. How do the conditions of space affect circadian rhythms and what roles do age and gender play?

2. What are the appropriate countermeasures for microgravity and the optimal environmental
3. What are the appropriate ground simulation models for studying the effects of the space environment on circadian rhythms?

4. What are the effects of the space environment on the interaction between the circadian and reproductive systems?

5. What are the effects of cephalad fluid shifts on circadian rhythms?

Regulatory: Endocrinology

NRC SSB 98/Recommendations:

- ? ? Studies should continue to evaluate the relevance of ground-based models to spaceflight.

1. How do space-induced changes in endocrine system function affect other homeostatic systems?

2. What are the short and long-term effects of spaceflight on endocrine system homeostasis and responsiveness?

3. How does spaceflight affect hormone function?

4. How does prolonged or repeated spaceflight affect regulation of mammalian reproductive cycles and behavior, and circadian regulation of hormone secretion and function?

Regulatory: Fluid and Electrolyte Balance

NRC SSB 98/Recommendations:

? ? Further hematology research is not of the highest priority. As a result of the /sls-1 and SLS-2 studies, the hematological changes must be regarded as one of the best-understood physiological changes that occur during spaceflight. Where the opportunity is available, it would be useful to refine the details of these processes, particularly that of sequestration in bone.

? ? Investigations of how erythropoiesis is regulated by hyper- and hypogravity should be continued with human and rodent models.

1. What are the time course, magnitude and mechanism of fluid shifts and volume changes during acclimatization to microgravity and readaptation to 1-g?

2. How do the fluid and electrolyte regulating mechanisms influence the cardiovascular responses to microgravity?

3. What are the effects of microgravity on renal function?

Regulatory: Hematology

1. What is the underlying cause of the decrease in red blood cell mass that occurs in space?

2. What are the time courses and magnitude of changes in the erythropoietic system during spaceflight?

3. Should red cell mass be restored during spaceflight and if so, what are the appropriate measures?

4. Is the postflight recovery time course from "spaceflight anemia" similar to that which occurs on Earth from blood donations and can the recovery be accelerated?

Regulatory: Metabolism and Nutrition

1. How does spaceflight alter energy requirements? To what extent are basal metabolic rate and efficiency altered during extended spaceflight?

2. To what extent does spaceflight alter gastrointestinal or liver function?

3. Are there valid ground models and analogs for the study of the effects of spaceflight on metabolism and nutrition?

4. What are the effects of spaceflight on nutritional requirements and use of supplements?

Regulatory: Temperature Regulation

1. What are the independent and compounded effects of spaceflight and EVA on thermoregulation processes and heat exchange? What specific environmental conditions influence temperature regulation?

2. What are the effects of prescribed countermeasures on thermoregulation?

3. How are changes in body temperature or its regulation correlated with metabolic rate, energy expenditure, and altered hormonal status?

5. What are the gender differences in temperature regulation as influenced by microgravity exposure?

RECOMMENDATIONS FOR RESEARCH PRIORITIES*

From the NRC Space Studies Board report "A Strategy for research in Space Biology and Medicine in the New Century

* Topics are not presented in an order or priority

I. PHYSIOLOGICAL AND PSYCHOLOGICAL EFFECTS OF SPACEFLIGHT

Loss of Weight-Bearing Bone and Muscle

- Research should emphasize studies that provide mechanistic insights into the development of effective countermeasures for preventing bone and muscle deterioration during and after spaceflight.
- Ground-based model systems, such as hindlimb unloading in rodents, should be used to investigate the mechanisms of changes that reproduce in-flight and post-flight effects.
- A database on the course of microgravity-related bone loss and its reversibility in humans should be established in pre-flight, in-flight, and post-flight recording of bone mineral density.

Vestibular Function, the Vestibular Ocular Reflex, and Sensorimotor Integration

- Experiments to determine the basis for the compensation on Earth and in space, and to evaluate whether the mechanisms are the same, should receive the highest priority, since these compensatory mechanisms operate in astronauts entering and returning from space and may have a profound effect on their performance in space and their postflight recovery on Earth.
- In-flight recordings of signal processing following otolith afferent stimulation should be made by a trained physiologist serving as a payload specialist, to determine how exposure to microgravity affects central and vestibular function and development.
- From ground-based experiments, we know that the vestibulo-oculomotor system is capable of learning new motor patterns in response to sensory perturbations; therefore, future investigation should focus on determining whether and how these mechanisms are affected by exposure to microgravity.

Orthostatic Intolerance Upon Return to Earth Gravity

- Current knowledge of the magnitude, time course, and mechanisms of cardiovascular adjustments should be extended to include long-duration exposure to microgravity.
- The specific mechanisms underlying inadequate total peripheral resistance observed during post-flight orthostatic stress should be determined.
- Current antiorthostatic countermeasures should be reevaluated to refine those that offer protection and eliminate those that do not. Studies should avoid confounding effects of multiple, simultaneous interventions unless data support these combinations. Priority should be given to interventions that may provide simultaneous bone and/or muscle protection.

- ? ? Appropriate methods for referencing intrathoracic vascular pressures to systemic pressures in microgravity should be identified and validated, given the observed changes in cardiac and pulmonary volume and compliance.

Radiation Hazards

- ? ? Determine the carcinogenic risks following irradiation by protons and high-atomic-number, high-energy (HZE) particles.
- ? ? Determine if exposure to heavy ions at the level that would occur during deep-space missions of long duration poses a risk to the integrity and function of the central nervous system.
- ? ? Determine whether combined effects of radiation and stress on the immune system in spaceflight could produce additive or synergistic effects on host defenses.

Physiological Effects of Stress

- ? ? Interactions between the hypothalamic-pituitary-adrenal (HPA) axis and the immune system during spaceflight should be analyzed to determine the role that the host response to stressors plays in alterations in host defenses.

Psychological and Social Issues

Not directly applicable to non-human life sciences research.

? ? *FUNDAMENTAL GRAVITATIONAL BIOLOGY*

Mechanisms of Graviperception and Gravitropism in Plants

- ? ? Studies of graviperception should concentrate on three problems:
- ? ? The identity of the cells that perceive gravity in multicellular plants;
- ? ? The intracellular mechanisms by which the direction of the gravity vector is perceived; and,
- ? ? The threshold value for graviperception – this will require a spaceflight experiment.
- ? ? Studies of gravitropic transduction should focus on the nature of the cellular asymmetry that is set up in a cell that perceives the direction of the gravity vector.
- ? ? Studies on the translocation step should concentrate on the nature and mechanism of the translocation of the signals that pass from the site of perception to the site or reaction.
- ? ? Studies on the reaction step should focus on the mechanism(s) by which gravitropic signals cause unequal rates of cell elongation, and on the possible effects of gravity on the sensitivity of these cells to the signals.

Mechanisms of Graviperception in Animals

8. Space-based experiments are needed to test the role of gravity on the embryonic development and maintenance of the vertebrate vestibular system. Prior to this, ground-based studies are needed to identify the critical periods in vestibular neuron development. In both Earth and space-based studies, it is important to characterize vestibular development at several different times to determine the sequence of intermediate events leading up to the final outcome. In space studies, controls for the effects of nongravitational stresses, including loud noise and vibration, must be performed on the ground so that the space experiments are designed to isolate the effects of microgravity from the effects due to other stresses.

Effects of Spaceflight on Reproduction and Development

- ? ? Key model animals should be grown through two life cycles; highest priority should be given to vertebrate models (e.g., fish, birds, and small mammals such as mice or rats). If significant developmental effects are detected, control experiments must be performed (including the use of a space-based 1-g centrifuge) to determine whether gravity or some other element of the space environment induces these developmental abnormalities.
- ? ? An analogous experiment should be carried out with the model plant *Arapidopsis thaliana* to confirm results obtained on Mir with a preliminary experiment using *Brassica rapa*. The ideal experiment will require the development of a suitable plant growth unit and of *Arapidopsis* plants containing stress-indicator genes and/or mutations conferring insensitivity to environmental stresses.